



US009546396B2

(12) **United States Patent**  
**Cox et al.**

(10) **Patent No.:** **US 9,546,396 B2**  
(45) **Date of Patent:** **\*Jan. 17, 2017**

(54) **METHOD FOR DNA DOUBLE-STRAND  
BREAK REPAIR IN VITRO AND  
APPLICATIONS THEREOF**

(58) **Field of Classification Search**

None

See application file for complete search history.

(71) Applicants: **Wisconsin Alumni Research  
Foundation**, Madison, WI (US); **Board  
of Supervisors of Louisiana State  
University and Agricultural &  
Mechanical College**, Baton Rouge, LA  
(US)

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,333,178 B1 12/2001 Livneh et al.  
2012/0082990 A1 4/2012 Piepenburg et al.

OTHER PUBLICATIONS

(72) Inventors: **Michael M. Cox**, Madison, WI (US);  
**John R. Battista**, Baton Rouge, LA  
(US); **Audrey J. Klingele**, Madison, WI  
(US); **Joseph R. Piechura**, Hartford,  
WI (US)

d'Abbadie M. et al., "Molecular breeding of polymerases for  
amplification of ancient DNA", *Nat Biotechnol.*, 2007, 25 (8):939-  
943.

Blow N., "Exploring unseen communities", *Nature*, 2008, 453:687-  
690.

Butler J.M., et al., "Genetics and Genomics of Core Short Tandem  
Repeat Loci Used in Human Identity Testing", *J Forensic Sci.*, 2006,  
51 (2):253-265.

Warnecke F., et al., "Building on basic metagenomics with comple-  
mentary technologies", *Genome Biol.*, 2007, 8 (12):231.

Xu L., et al., "A Dynamic RecA Filament Permits DNA Polymerase-  
catalyzed Extension of the Invading Strand in Recombination  
Intermediates", *J Biol Chem.*, 2002, 277(16):14321-14328.

Burbano H.A., et al., "Targeted Investigation of the Neandertal  
Genome by Array-Based Sequence Capture", *Science*, 2010,  
328(5979):723-725.

Cox M.M., et al. "Rising from the Ashes: DNA Repair in *Deinococ-  
cus radiodurans*", *PLoS Genet.*, 2010, 6(1 ):e1 000815.

PCT Notification of Transmittal. International Search Report. and  
Written Opinion, PCT/US201 0/037793, Oct. 11, 2010.

Cox, Michael M., *Recombinational DNA Repair in Bacteria and the  
RecA Protein*, *Progress in Nucleic Acid Research and Molecular  
Biology*, 2000, vol. 63, pp. 311-366.

Kowalczykowski, Stephen C., *Initiation of Genetic Recombination  
and Recombination-Dependent Replication*, *Trends in Biochemical  
Sciences*, Apr. 2000, vol. 25, No. 4, pp. 156-165.

Pham, Phuong, et al., *Roles of DNA Polymerases V and II in  
SOS-Induced Error-Prone and Error-Free Repair in Escherichia  
Coli*, Jul. 2001, PNAS, vol. 98, No. 15, pp. 8350-8354.

European Patent Office, First Examination Report, Application No.  
10723897.4, Apr. 4, 2014.

*Primary Examiner* — Nancy J Leith

(74) *Attorney, Agent, or Firm* — Quarles & Brady LLP

(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 63 days.

This patent is subject to a terminal dis-  
claimer.

(21) Appl. No.: **14/066,126**

(22) Filed: **Oct. 29, 2013**

(65) **Prior Publication Data**

US 2014/0134628 A1 May 15, 2014

**Related U.S. Application Data**

(62) Division of application No. 12/796,258, filed on Jun.  
8, 2010, now Pat. No. 8,586,362.

(60) Provisional application No. 61/268,255, filed on Jun.  
10, 2009, provisional application No. 61/185,868,  
filed on Jun. 10, 2009.

(51) **Int. Cl.**  
**C12Q 1/68** (2006.01)  
**C12N 15/00** (2006.01)

(52) **U.S. Cl.**  
CPC ..... **C12Q 1/6806** (2013.01); **C12Q 1/6844**  
(2013.01)

(57) **ABSTRACT**

A system and a method for DNA double-strand break repair  
in vitro are disclosed. Applications of the disclosed method  
in multiple areas are also disclosed.

**11 Claims, 11 Drawing Sheets**  
**(7 of 11 Drawing Sheet(s) Filed in Color)**